

## SEQUENCE PROTOCOL

&lt;110&gt; Degussa AG

5 <120> Process for the fermentative preparation of  
L-amino acids using strains of the  
Enterobacteriaceae family.

10 &lt;130&gt; 000425 BT

&lt;140&gt;

&lt;141&gt;

15 &lt;160&gt; 19

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 1622

20 &lt;212&gt; DNA

&lt;213&gt; Escherichia coli

&lt;220&gt;

&lt;221&gt; CDS

25 &lt;222&gt; (1)..(1620)

&lt;223&gt; pckA

&lt;400&gt; 1

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Met Arg Val Asn Asn Gly Leu Thr Pro Gln Glu Leu Glu Ala Tyr Gly  
1 5 10 15

35 atc agt gac gta cat gat atc gtt tac aac cca agc tac gac ctg ctg 96  
Ile Ser Asp Val His Asp Ile Val Tyr Asn Pro Ser Tyr Asp Leu Leu  
20 25 30

40 tat cag gaa gag ctc gat ccg agc ctg aca ggt tat gag cgc ggg gtg 144  
Tyr Gln Glu Glu Leu Asp Pro Ser Leu Thr Gly Tyr Glu Arg Gly Val  
35 40 45

tta act aat ctg ggt gcc gtt gcc gtc gat acc ggg atc ttc acc ggt 192  
Leu Thr Asn Leu Gly Ala Val Ala Val Asp Thr Gly Ile Phe Thr Gly  
50 55 60

45 cgt tca cca aaa gat aag tat atc gtc cgt gac gat acc act cgc gat 240  
Arg Ser Pro Lys Asp Lys Tyr Ile Val Arg Asp Asp Thr Thr Arg Asp  
65 70 75 80

50 act ttc tgg tgg gca gac aaa ggc aaa ggt aag aac gac aac aaa cct 288  
Thr Phe Trp Trp Ala Asp Lys Gly Lys Gly Lys Asn Asp Asn Lys Pro  
85 90 95

55 ctc tct ccg gaa acc tgg cag cat ctg aaa ggc ctg gtg acc agg cag 336  
Leu Ser Pro Glu Thr Trp Gln His Leu Lys Gly Leu Val Thr Arg Gln  
100 105 110

60 ctt tcc ggc aaa cgt ctg ttc gtt gtc gac gct ttc tgt ggt gcg aac 384  
Leu Ser Gly Lys Arg Leu Phe Val Val Asp Ala Phe Cys Gly Ala Asn  
115 120 125

T04250-9992560

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	Pro Asp Thr Arg Leu Ser Val Arg Phe Ile Thr Glu Val Ala Trp Gln	
	130 135 140	
5	gcg cat ttt gtc aaa aac atg ttt att cgc ccg agc gat gaa gaa ctg	480
	Ala His Phe Val Lys Asn Met Phe Ile Arg Pro Ser Asp Glu Glu Leu	
	145 150 155 160	
10	gca ggt ttc aaa cca gac ttt atc gtt atg aac ggc gcg aag tgc act	528
	Ala Gly Phe Lys Pro Asp Phe Ile Val Met Asn Gly Ala Lys Cys Thr	
	165 170 175	
15	aac ccg cag tgg aaa gaa cag ggt ctc aac tcc gaa aac ttc gtg gcg	576
	Asn Pro Gln Trp Lys Glu Gln Gly Leu Asn Ser Glu Asn Phe Val Ala	
	180 185 190	
20	ttt aac ctg acc gag cgc atg cag ctg att ggc ggc acc tgg tac ggc	624
	Phe Asn Leu Thr Glu Arg Met Gln Leu Ile Gly Gly Thr Trp Tyr Gly	
	195 200 205	
25	ggc gaa atg aag aaa ggg atg ttc tcg atg atg aac tac ctg ctg ccg	672
	Gly Glu Met Lys Lys Gly Met Phe Ser Met Met Asn Tyr Leu Leu Pro	
	210 215 220	
30	ctg aaa ggt atc gct tct atg cac tgc tcc gcc aac gtt ggt gag aaa	720
	Leu Lys Gly Ile Ala Ser Met His Cys Ser Ala Asn Val Gly Glu Lys	
	225 230 235 240	
35	ggc gat gtt gcg gtg ttc ttc ggc ctt tcc ggc acc ggt aaa acc acc	768
	Gly Asp Val Ala Val Phe Phe Gly Leu Ser Gly Thr Gly Lys Thr Thr	
	245 250 255	
40	ctt tcc acc gac ccg aaa cgt cgc ctg att ggc gat gac gaa cac ggc	816
	Leu Ser Thr Asp Pro Lys Arg Arg Leu Ile Gly Asp Asp Glu His Gly	
	260 265 270	
45	tgg gac gat gac ggc gtg ttt aac ttc gaa ggc ggc tgc tac gca aaa	864
	Trp Asp Asp Asp Gly Val Phe Asn Phe Glu Gly Gly Cys Tyr Ala Lys	
	275 280 285	
50	act atc aag ctg tcg aaa gaa gcg gaa cct gaa atc tac aac gct atc	912
	Thr Ile Lys Leu Ser Lys Glu Ala Glu Pro Glu Ile Tyr Asn Ala Ile	
	290 295 300	
55	cgt cgt gat gcg ttg ctg gaa aac gtc acc gtg cgt gaa gat ggc act	960
	Arg Arg Asp Ala Leu Leu Glu Asn Val Thr Val Arg Glu Asp Gly Thr	
	305 310 315 320	
60	atc gac ttt gat gat ggt tca aaa acc gag aac acc cgc gtt tct tat	1008
	Ile Asp Phe Asp Asp Gly Ser Lys Thr Glu Asn Thr Arg Val Ser Tyr	
	325 330 335	
65	ccg atc tat cac atc gat aac att gtt aag ccg gtt tcc aaa gcg ggc	1056
	Pro Ile Tyr His Ile Asp Asn Ile Val Lys Pro Val Ser Lys Ala Gly	
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70	cac gcg act aag gtt atc ttc ctg act gct gat gct ttc ggc gtg ttg	1104
	His Ala Thr Lys Val Ile Phe Leu Thr Ala Asp Ala Phe Gly Val Leu	
	355 360 365	

000425 BT / AL1

ccg ccg gtt tct cgc ctg act gcc gat caa acc cag tat cac ttc ctc 1152  
 Pro Pro Val Ser Arg Leu Thr Ala Asp Gln Thr Gln Tyr His Phe Leu  
 370 375 380

5 tct ggc ttc acc gcc aaa ctg gcc ggt act gag cgt ggc atc acc gaa 1200  
 Ser Gly Phe Thr Ala Lys Leu Ala Gly Thr Glu Arg Gly Ile Thr Glu  
 385 390 395 400

10 ccg acg cca acc ttc tcc gct tgc ttc ggc gcg gca ttc ctg tgc ctg 1248  
 Pro Thr Pro Thr Phe Ser Ala Cys Phe Gly Ala Ala Phe Leu Ser Leu  
 405 410 415

15 cac ccg act cag tac gca gaa gtg ctg gtg aaa cgt atg cag gcg gcg 1296  
 His Pro Thr Gln Tyr Ala Glu Val Leu Val Lys Arg Met Gln Ala Ala  
 420 425 430

20 ggc gcg cag gct tat ctg gtt aac act ggc tgg aac ggc act ggc aaa 1344  
 Gly Ala Gln Ala Tyr Leu Val Asn Thr Gly Trp Asn Gly Thr Gly Lys  
 435 440 445

cgt atc tgc att aaa gat acc cgc gcc att atc gac gcc atc ctc aac 1392  
 Arg Ile Ser Ile Lys Asp Thr Arg Ala Ile Ile Asp Ala Ile Leu Asn  
 450 455 460

25 ggt tgc ctg gat aat gca gaa acc ttc act ctg ccg atg ttt aac ctg 1440  
 Gly Ser Leu Asp Asn Ala Glu Thr Phe Thr Leu Pro Met Phe Asn Leu  
 465 470 475 480

30 gcg atc cca acc gaa ctg ccg gcc gta gac acg aag att ctc gat ccg 1488  
 Ala Ile Pro Thr Glu Leu Pro Gly Val Asp Thr Lys Ile Leu Asp Pro  
 485 490 495

35 cgt aac acc tac gct tct ccg gaa cag tgg cag gaa aaa gcc gaa acc 1536  
 Arg Asn Thr Tyr Ala Ser Pro Glu Gln Trp Gln Glu Lys Ala Glu Thr  
 500 505 510

40 ctg gcg aaa ctg ttt atc gac aac ttc gat aaa tac acc gac acc cct 1584  
 Leu Ala Lys Leu Phe Ile Asp Asn Phe Asp Lys Tyr Thr Asp Thr Pro  
 515 520 525

gcg ggt gcc gcg ctg gta gcg gct ggt ccg aaa ctg taa 1623  
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 530 535 540

45 <210> 2  
 <211> 540  
 <212> PRT  
 <213> Escherichia coli

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Tyr Gln Glu Glu Leu Asp Pro Ser Leu Thr Gly Tyr Glu Arg Gly Val  
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[illegible]

[illegible]

<222> (543)..(1105)

<223> Part of the 3' region (pck2) of the pckA gene

<220>

5 <221> misc\_feature

<222> (1106)..(1156)

<223> Technical DNA/residues of the polylinker sequence

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 caccaaaaga taagtatatc gtccgtgacg ataccactcg cgatactttc tgggtgggcag 180  
 acaaaggcaa aggttaagaac gacaacaaac ctctctctcc ggaaacctgg cagcatctga 240  
 aaggcctggt gaccaggcag ctttccggca aacgtctgtt cgttgtcgac gctttctgtg 300  
 15 gtgcgaaccc ggatactcgt ctttccgtcc gtttcatcac cgaagtggcc tggcaggcgc 360  
 attttgtcaa aaacatgttt attcgcccga gcgatgaaga actggcaggt ttcaaaccag 420  
 actttatcgt tatgaacggc gcgaagtgca ctaaccgcga gtggaaagaa cagggtctca 480  
 actccgaaaaa cttcgtggcg tttaacctga ccgagcgcat gcaagccgaa ttctgcagat 540  
 cctgaagatg gcactatcga ctttgatgat ggttcaaaaa ccgagaacac ccgcgtttct 600  
 20 tatccgatct atcacatcga taacattgtt aagccggttt ccaaagcggg ccacgcgact 660  
 aaggttatct tcctgactgc tgatgctttc ggcggtgtgc cgccggtttc tcgcctgact 720  
 gccgatcaaa ccagtatca cttcctctct ggcttcaccg ccaaactggc cggtactgag 780  
 cgtggcatca ccgaaccgac gccaaccttc tccgcttgct tcggcgcggc attcctgtcg 840  
 ctgcacccga ctacgtacgc agaagtgtg gtgaaacgta tgcaggcggc gggcgcgcgag 900  
 25 gcttatctgg ttaacactgg ctggaacggc actggcaaac gtatctcgat taaagatacc 960  
 cgcgccatta tcgacgccat cctcaacggg tcgctggata atgcagaaac cttoactctg 1020  
 ccgatgttta acctggcgat cccaaccgaa ctgccgggcg tagacacgaa gattctcgat 1080  
 ccgcgtaaca cctacgcttc tccggaagcc gaattctgca gatatccatc acactggcgg 1140  
 30 ccgctcgagc atgcat 1156

<210> 4

<211> 1294

<212> DNA

35 <213> Escherichia coli

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<222> (1)..(3)

40 <223> Start codon of the delta pckA allele

<220>

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<222> (1)..(598)

45 <223> 5' region of the delta pckA allele

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<222> (599)..(618)

50 <223> Technical DNA/residues of the polylinker sequence

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<222> (619)..(1291)

55 <223> 3' region of the delta pckA allele

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<221> misc\_feature

<222> (1292)..(1294)

60 <223> Stop codon of the delta pckA allele

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	ctgacagggtt	atgagcgcg	ggtgttaact	aatctgggtg	ccgttgccgt	cgataccggg	180
5	atcttcaccg	gtcgttcacc	aaaagataag	tatatcgcc	gtgacgatac	cactcgcgat	240
	actttctggt	gggcagacaa	aggcaaagg	aagaacgaca	acaaacctct	ctctccgga	300
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	gccgaattct	cgagatcctg	aagatggcac	tatcgacttt	gatgatgggt	caaaaaccga	660
	gaacacccgc	gtttcttatt	cgatctatca	catcgataac	attgttaagc	cggtttccaa	720
	agcgggcccac	gcgactaagg	ttatcttcct	gactgctgat	gctttcggcg	tgttgccgcc	780
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	cgaacacctg	gcgaaactgt	ttatcgacaa	cttcgataaa	tacacgcgaca	cccctgcggg	1260
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35	<220>						
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	<222> Complement((461)..(727))						
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	ccagattgtg	ggtaaaatcg	gcgagacgtt	tggcgtaagc	aatttagcgc	tcgacaccca	180
	gggagtaggc	gactcctccc	aggtagtggt	cagcggctat	gtattgccag	gtctgcaagt	240
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	gcctaagcta	tatctggaag	ccgtgtctgg	tgtagaccag	gcactggatt	tgtcttatca	360
	gttcgagttt	tagcaatgcg	aataattgtc	tacggcagtt	tacgccacaa	acaaggcaac	420
	agtcactgga	tgaccaatgc	ccagttactg	ggcgatttca	gtatcgataa	ctaccagttg	480
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	atactttgta	taacttaagg	aggtgcagat	gcgtattacc	ataaaaagat	gggggaacag	960
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 20 <223> 3' flank of the ytfP-yjfa region  
  
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 <222> (376)..(378)  
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 30 <223> ATG codon of the truncated ORF yjfa  
  
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 35 ccagattgtg ggtaaaatcg gcgagacgtt tggcgtaagc aatttagcgc tcgacacca 180  
 gggagtaggc gactcctccc aggtagtggc cagcggctat gtattgccag gtctgcaagt 240  
 gaaatacggc gtgggtatat ttgactctat agcaacactc acgttacgtt atcgctgat 300  
 gcctaagcta tatctggaag ccgtgtcttg ttagaccag gcaactggatt tgctctatca 360  
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 40 gagacgactc gcattctgtt ttgtaattcc ctcacctttt gcttttctct ccgagccgct 480  
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 45 acacccatct ccaggcgcta ctgccttgat gaactgctgg cacagtgtga catgaacgcc 780  
 gcggaactta gcgagcagga tgtctggggc aaatccaccc ctgcgggtga cgaaatatgg 840  
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 <221> misc\_feature

000000-000000



<222> (1)..(630)  
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 5 <221> misc\_feature  
 <222> (631)..(1158)  
 <223> 3' flank of the ytfP-yjfa region

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 10 <221> misc\_feature  
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 15 <221> misc\_feature  
 <222> Complement((635)..(637))  
 <223> ATG codon of the truncated ORF yjfa

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 tcagagcgac agtgcggcaa tgacctcgat gctgattggg ttggggggtg cgcaaagtgg 120  
 ccagattgtg ggtaaaatcg gcgagacgtt tggcgtaagc aatttagcgc tcgacaccca 180  
 gggagtaggc gactcctccc aggtagtggg cagcggctat gtattgccag gtctgcaagt 240  
 gaaatacggc gtgggtatat ttgactctat agcaacactc acgttacgtt atcgccctgat 300  
 25 gcctaagcta tatctggaag ccgtgtctgg ttagaccag gcactggatt tgctctatca 360  
 gttcgagttt tagcaatgcg aatatttgtc tacggcagtt tacgccacaa acaaggcaac 420  
 agtcactgga tgaccaatgc ccagttactg ggcgatttca gtatcgataa ctaccagttg 480  
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 ggtaaaacgt ttctgtagc accgtgagtt atactttgta taacttaagg aggtgcagat 840  
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 45 <213> Artificial sequence

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 <223> Description of the artificial sequence: Primer  
 pckA'5'-1

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 30 ccggagaagc gtaggtgtta 20  
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 55 <210> 14  
 <211> 20  
 <212> DNA  
 <213> Artificial sequence  
 60 <220>  
 <223> Description of the artificial sequence: Primer RhtC1

000425 BT / AL1  
 10  
 5  
 20  
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 30  
 35  
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